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SOME EFFECTS OF STIMULATION OF SYMPATHETIC NERVES AND INJECTION OF PRESSOR DRUGS IN ADRENALECTOMIZED CATS

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In 1904 Elliott reported that in cats dying of adrenal insufficiency the tissues innervated by the sympathetic failed to respond to electrical stimulation of their nerves or to nicotine. He suggested that the presence of adrenaline, or an immediate precursor, was necessary for the excitation of the peripheral tissue by the sympathetic and postulated that adrenaline might be the stimulating agent liberated by sympathetic nerve impulses.

Further light on the actual site of this functional defect in the sympathetic nerves was provided by Elliott [1914] in his analysis of the nature of death in adrenal insufficiency. In cats prostrate and at the point of death he found no evidence of disturbance of somatic, parasympathetic or visceral afferent nerves. The sympathetic vasoconstrictor nerves, however, seemed paralysed, at least in the splanchnic region, for stimulation of these nerves led to little or no rise in blood pressure. On the other hand, a minimal stimulus of the cervical sympathetic produced dilatation of the pupil. Intravenously injected nicotine had a negligible pressor effect, which implied that cardio-accelerator and vasoconstrictor fibres other than those in the splanchnics were functionally defective. Since pituitrin and barium chloride also failed to produce any substantial increase in blood pressure, it seemed that the paralysis was due to a loss in the power of contractility of vascular muscle and not necessarily to a defect in the nerves. Adrenaline, however, still produced substantially normal cardiovascular effects, indicating that the muscle was still capable of contraction. This analysis suggests, in Elliott's words, that

"the nerves appear to be paralysed because the muscle cannot respond to their excitatory impulses". It could be further explained by postulating a failure of the sympathetic vasoconstrictor and cardio-accelerator nerves to liberate an effective concentration of the hypothetical stimulating agent suggested by him in 1904. Such a postulate appears reasonable in the light of the recent confirmation of the hypothesis of chemical transmission of autonomic nerve impulses supplied by the work of Loewi, Cannon, Dale and their collaborators. At adrenergic nerve endings (using the nomenclature of Dale [1933]) the chemical transmitter appears to be remarkably like, if not identical with adrenaline. Detailed consideration of the literature on this subject is unnecessary here in view of the thorough reviews of Bacq [1935] and of Eccles [1936], but it is pertinent to note that the sympathetic failure noted by Elliott in adrenal insufficiency appeared to concern the adrenergic and not the cholinergic vasomotor fibres of the autonomic system.

Other reports and views concerning the nature of the cardiovascular failure in adrenal insufficiency have been considered in the reviews of Hoskins [1922] and of Britton [1930]. The former author concluded that the hypotension following adrenalectomy was due to a defect in the effector rather than in the sympathetic system. The latter cited particularly the marked concentration of the blood and the conclusion of Coombs [1925] that some product of the adrenals is necessary for the maintenance of sympathetic nerve activity on vascular smooth muscle. Observations of Langsdorf [1933] indicate a diminished sympathetic tone in adrenalectomized rabbits. A significant study by Swingle, Pfiffner, Vars & Parkins [1934] showed that adrenalectomized dogs have lost the power to dilute their own blood after haemorrhage. More recently, Swingle, Parkins, Taylor & Hays [1937] and Parkins, Swingle, Taylor & Hays [1938] have concluded in addition that adrenal cortical hormone has a pressor effect separate from its action on a blood diluting mechanism. The function of sympathetic nerves immediately following removal of the adrenals has been studied by Secker [1938].

While the vasomotor paralysis by Elliott may have been secondary to the moribund condition of the animals at the time of the experiments, his findings do not seem to have been examined critically in detail. In view of this we felt the problem worthy of reinvestigation, and in this paper shall describe the results obtained from experiments along the lines of those by which he investigated the function of sympathetic nerves and the effect of drugs on the cardiovascular system of cats dying of adrenal insufficiency.

Methods

Cats were used throughout. In one group of experiments Elliott's experimental conditions were faithfully observed; ether was used as the anaesthetic, and the splanchnics stimulated in the open thorax while artificial respiration was maintained by a pump. It was found, however, that the prostrated animal frequently died during ether anaesthesia or following the trauma involved in opening the thorax. In some experiments (e.g. Exp. 4), therefore, a modified technique was employed: nembutal was used as the anaesthetic and the great splanchnic was stimulated in the abdomen. This modification did not seem to prejudice the results.

Adrenalectomy generally was performed by the lumbar route in two stages. In most instances adrenal cortical extract was administered following removal of the second gland. This was injected subcutaneously twice daily for 3 or 4 days in 3–5 c.c. doses, and thereafter in half this amount until discontinued in order to allow the development of adrenal insufficiency. In some cases both glands were removed at one operation through a right rectus incision. The occasional occurrence of sepsis was successfully combated by surgical measures and increased amounts of extract. When upper respiratory infection occurred, however, it was but rarely overcome by the injection of even large quantities of extract.

The cats were kept in a room where the temperature varied little from 72° F. A vermifuge was administered routinely. The diet consisted of milk, canned salmon and, occasionally, cooked meat and chicken. The results considered for this paper, with a few exceptions, are those obtained on animals which received extract and did well on the injections. The criterion utilized was that they should weigh more at the time the extract was discontinued than at the time of the operation.

For stimulation of sympathetic nerves a Harvard inductorium was used, which had a spring vibrator with a frequency of 32 c./sec. in the circuit. Both make and break shocks were effective and the stimulus supramaximal when the secondary coil distance was 6 cm. In the experiments, when not stated otherwise, the coil distance is 6 cm.

The contractions of the nictitating membrane (n.m.) were recorded isotonically, a lever with a ten- to fifteen-fold magnification and a tension of 4 g. being used. Carotid blood pressure was recorded by a mercury manometer. Time on the tracings is marked in 10 sec. intervals.

Drugs, except where otherwise stated, were injected by the femoral vein in constant volume (1 c.c.) over a 10 sec. interval. Parke, Davis and

Co. pitressin was used except where otherwise indicated. The usual dose of this drug was 2 units (0·2 c.c.) made up to 1 c.c. with normal saline. The concentration of barium chloride injected was 2·5 %. Blood for analysis was generally drawn from the femoral vein at the start of the experiment.

The adrenal cortical extract used was prepared in the Connaught Laboratories, Toronto, and has been reported on by Cleghorn, McHenry, McVicar & Overend [1937]. This material shows none of the toxicity of an earlier preparation [Cleghorn, 1932].

Blood sodium was determined according to the principles of the method of Butler & Tuthill [1931] on either serum or plasma. When sodium was estimated on plasma, heparin was used as the anticoagulant. The technique developed, permitting the use of as little as 0·5 c.c. of serum, is as follows: The material was first wet-ashed in a pyrex test-tube with sulphuric and nitric acids until the fluid was quite clear and sulphuric oxide fumes evolved. The contents of the test-tube were then transferred to a beaker, quantitatively, by means of four washings of uranium zinc acetate solution, giving a volume of 12–15 c.c. Precipitation of the sodium salt takes place in the ensuing thirty minutes, filtration, washing and weighing of the precipitate being carried out as described by Butler & Tuthill. Determinations were done in duplicate.

Potassium analyses were carried out according to the method of Shohl & Bennett [1928].

Non-protein nitrogen. Blood was digested in a silica test-tube with phosphoric sulphuric digestion mixture, the product being directly nesslerized and determined by colorimetric comparison with a standard ammonium chloride solution similarly nesslerized.

The term *sympathin* [Cannon & Bacq, 1931] will be used to designate the adrenaline-like mediator of adrenergic nerve impulses, though the significance of this term, as later extended by Cannon & Rosenblueth [1933], has not met with general acceptance [Bacq, 1935; Eccles, 1936; Loewi, 1936].

The following abbreviations will be employed in the description of the experiments and legends of this paper: a.c.e. adrenal cortical extract; stim. stimulation or stimulated.

Results

(1) *Examination of cats in the terminal stage of adrenal insufficiency*

Signs of adrenal insufficiency, in order of their appearance, are loss of appetite, apathy and weakness. Weakness, in animals surviving up to two weeks after adrenalectomy or cessation of extract injections, may be

apparent a day or two before death occurs. However, we have found that the blood pressure is but little below normal in animals in the early stage of weakness. The progress of weakness to the state of staggering indicates the onset of what we arbitrarily call the terminal stage of adrenal insufficiency. Except in occasional instances in which this is due to severe hypoglycaemia, it is associated with marked reduction in blood pressure and vascular failure is then demonstrable. The period of staggering is associated with a rapid fall in rectal temperature. It lasts a few hours, merging into the state of prostration when the animal is too weak to rise.

(*a*) *Vascular failure.* One of a group of six experiments carried out under Elliott's conditions is described in Exp. 1.

Experiment 1. *Cat* 62♀

16. xi. 38. Both adrenal glands removed. 2·21 kg. Received no adrenal cortical extract.

25. xi. 38. Almost prostrate; staggers badly if placed on feet, then sinks to floor in unnatural position. Weight 2·1 kg. Rectal temp. 33° C. Etherized. B.P. 45 mm. Hg, falling to 24 mm. after section of splanchnics in thorax. Both splanchnics stim.: no effect

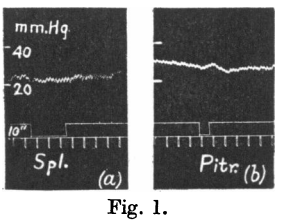

Fig. 1.

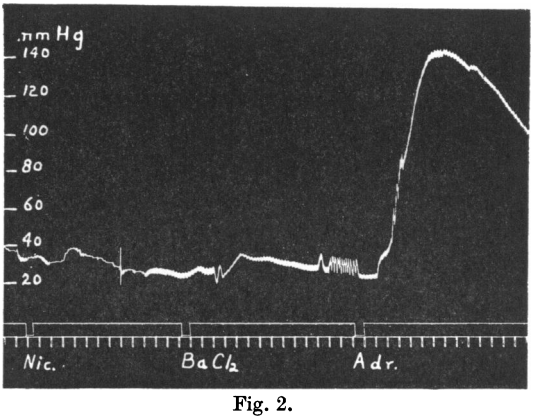

Fig. 2.

Fig. 1. Exp. 1. Carotid blood pressure of an adrenalectomized cat (and in all other tracings). Ether. Terminal stage adrenal insufficiency. (*a*) *Spl.* both splanchnics stimulated in thorax. (*b*) *Pitr.* 2 units pitressin injected intravenously.

Fig. 2. Exp. 1. Continuation of tracing in Fig. 1. *Nic.* 10 mg. nicotine injected. $BaCl_2$, 25 mg. barium chloride injected. *Adr.* 0·05 mg. adrenaline injected.

(Fig. 1*a*). Adrenaline 0·05 mg. injected: B.P. rose from 30 to 94 mm. Hg. Pitressin 2 units injected: questionable effect (Fig. 1*b*). Both splanchnics stim. again: no effect. Adrenaline 0·05 mg. injected: B.P. rose from 22 to 110 mm. Hg. Atropine (bellafoline) 1 mg. injected. Adrenaline 0·05 mg. injected 1 min. later: B.P. rose from 31 to 124 mm. Hg. Nicotine 10 mg. injected 8 min. after atropine: B.P. changed 36—34—39 (Fig. 2). $BaCl_2$ 25 mg. injected: B.P. rose from 27 to 38 mm.; fell quickly (Fig. 2). Adrenaline 0·05 mg. injected: B.P. rose

from 26 to 146 mm. Hg (Fig. 2). Blood drawn: haematocrit 47%; potassium 34·3 mg./100 c.c.; non-protein nitrogen 83·5 mg./100 c.c.

Autopsy. Small area of infection at upper end of abdominal incision about 1 cm. in diameter. Pancreas congested. Gastric mucosa congested; two or three punctate ulcers near pylorus. Duodenum and ileum show congested mucosa. No residual cortical tissue found.

While these results are similar to those obtained by Elliott, many of our cats examined in adrenal insufficiency (e.g. Exps. 2–4) do not show quite so marked a vascular paralysis as that observed by him. This probably is due to the fact that the experiments were begun slightly earlier in the decline of the animal, during the period of weakness that immediately precedes the shorter period of prostration. We intentionally adopted the expedient of beginning earlier since we had lost a number of animals by waiting for the period of prostration when death occurred on administration of the anaesthetic or from a failing circulation before any records could be obtained.

Exps. 1–4 should be compared with the results on controls in Table I and also with Exps. 5 and 6.

TABLE I. The blood-pressure rise in acutely* adrenalectomized cats in response to stimulation of the peripheral cut end of the right great splanchnic nerve in the abdomen and to the injection of various pressor drugs. Record of systolic blood pressure before each procedure and at point of maximal rise, in mm. Hg.

Cat† no.	Right splanchnic nerve stimulation 30 sec.	Adrenaline 0·02 mg.	Pitressin 2 units	Barium chloride 25 mg.
83	114–162	115–196	98–144	110–190
90‡	80–152	90–176	73–153	90–200
92	102–160	92–156	110–142	—
93	101–206	116–226	120–194	138–256
97	106–148	92–196	90–142	108–221

* Operation less than 1½ hr. prior to recorded observation.

† Anaesthetic, nembutal: except no. 93 which was under "dial".

‡ Cat in poor state of nutrition—thin.

It was desirable to eliminate the possibility that the shock of the operation of adrenalectomy or the occurrence of infection then acquired was the cause of the vascular paralysis. This was done by treating animals with cortical extract for a period after operation until the pre-operative weight had been regained and the wounds were obviously free of infection. Thereafter extract was withheld. In cats so treated symptoms of final collapse did not appear as early as in those which did not receive extract after operation. As shown by the following two experiments, the vascular paralysis observed, in the terminal stage was just as marked, however, as in the untreated cats at the same stage.

Experiment 2. *Cat* 9♀

7. x. 35. Second adrenal removed. Received daily injection of adrenal cortical extract for 6 days.

13. x. 35. Last day of injections. Weight 2·79 kg.

25. x. 35. Weak; gait staggering; weight 2·41 kg. Etherized: B.P. 85 mm. Hg, falling to 20 mm. after section of splanchnics in thorax. Adrenaline 0·02 mg. injected: B.P. rose from 20 to 60 mm. Hg. Both splanchnics stim. peripherally in thorax—coil distance 12 cm.: no effect. Adrenaline 0·02 mg. injected: B.P. rose from 20 to 74 mm. Hg. Left splanchnic stim.—coil distance 6 cm.: B.P. rose from 20 to 26 mm. Hg. Pitressin 2 units injected: B.P. rose from 20 to 42 mm. Hg, falling to 33 mm. 70 sec. later. Adrenaline 0·02 mg. injected: B.P. rose from 34 to 114 mm. Hg. Atropine 5 mg. injected: B.P. fell rapidly so more adrenaline injected. Nicotine 20 mg. injected 5 min. after atropine: B.P. rose from 34 to 44 mm. Hg. Pitressin 2 units injected: B.P. rose from 30 to 34 mm. Hg; fell after 30 sec. Adrenaline 0·02 mg. injected: B.P. rose from 23 to 76 mm. Hg. Autopsy revealed no residual cortical tissue. Blood: serum sodium 287 mg./100 c.c.

Experiment 3. *Cat* 11♂

1. x. 35. Both adrenals removed by abdominal route. Received adrenal cortical extract for 19 days.

20. x. 35. Weight 2·95 kg.

30. x. 35. Weak; gait staggering; weight 2·5 kg. Rectal temp. 35·2° C. Etherized: B.P. 52 mm. Hg. Left vagus stim. momentarily: heart slowed and B.P. fell 10 mm. Hg. Adrenaline 0·02 mg. injected: B.P. rose from 56 to 100 mm. Hg, returning to 56 and falling to

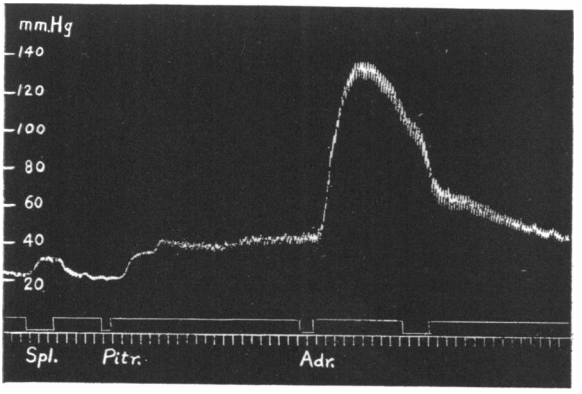

Fig. 3.

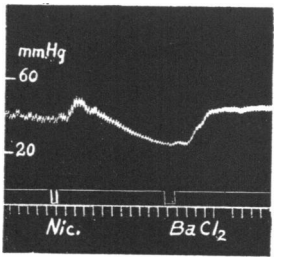

Fig. 4.

Fig. 3. Exp. 3. Ether. Terminal stage adrenal insufficiency. *Spl.* both splanchnics stim. in thorax. *Pitr.* 2 units pitressin injected. *Adr.* 0·04 mg. adrenaline injected. Last signal, atropine 5 mg. injected.

Fig. 4. Exp. 3. Continuation of tracing in Fig. 3. *Nic.* 20 mg. nicotine injected. $BaCl_2$, 25 mg. barium chloride injected.

24 mm. Hg after section of splanchnics in thorax. Both splanchnics stim.—coil distance 12 cm.: no effect. Stim. again—coil distance 10 cm.: no effect. Adrenaline 0·02 mg. injected: B.P. rose from 20 to 56 mm. Hg. Both splanchnics stim.—coil distance 8 cm.: no effect. Stim. again—coil distance 6 cm.: B.P. rose from 24 to 33 mm. (Fig. 3). Pitressin

2 units injected: B.P. rose from 23 to 42 mm. Hg (Fig. 3). Adrenaline 0·04 mg. injected: B.P. rose from 42 to 140 mm. Hg (Fig. 3). Atropine 5 mg. injected. Nicotine 20 mg. injected 4½ min. later: B.P. rose from 40 to 50 mm., falling in the next 2 min. to 26 mm. Hg (Fig. 4). Barium chloride 25 mg. injected: B.P. rose sharply from 26 to 46 mm. Hg and slowly to 53 mm. Hg (Fig. 4). Still 42 mm. Hg 12 min. later when next injection given. Pitressin 2 units injected: B.P. rose sharply from 42 to 48 mm. Hg; fell 20 sec. later to 34 mm. Hg, rising more slowly to 44 mm. Hg. Adrenaline 0·04 mg. injected: B.P. rose from 44 to 125 mm. Hg. Autopsy revealed no residual cortical tissue. Blood: serum sodium 315 mg./100 c.c.; non-protein nitrogen 123 mg./100 c.c.

(*b*) *Effectiveness of stimulation of the cervical sympathetic.* The defective response from sympathetic nerve stimulation, as Elliott pointed out, does not seem to be shared by those fibres supplying the eye. This has been confirmed by stimulation of the peripheral cut end of the cervical sympathetic (preganglionic) with resulting immediate dilatation of the pupil and powerful contraction of the nictitating membrane (n.m.). In two animals exhibiting signs of severe insufficiency the contraction of the n.m. was well sustained by continuous stimulation for more than two hours, though splanchnic nerve stimulation produced only a slight and briefly sustained rise in blood pressure in each case.

Experiment 4. *Cat* 19♀

16. xi. 35. Second adrenal removed. Received daily injections of adrenal cortical extract for 76 days.

31. i. 36. Last day of injections. 3·12 kg.; had gained 520 g.

13. ii. 36. Weak; can barely stand; 2·48 kg. Rectal temp. 31·1° C. Nembutal 40 mg. intravenously . Head fixed in Czermak holder. Right cervical sympathetic chain isolated and cut. Right n.m. attached to lever with 10-fold magnification and weight of 4 g. Peripheral end right cervical sympathetic stim. continuously—coil distance 6 cm.: n.m. contracted instantly, the writing point recording a rise of 55 mm. and pupil dilated. After 2 hr. stim. height of recorded contraction still 30 mm. and at 2½ hr., when stim. terminated, 10 mm. above starting level. Carotid cannula inserted: B.P. 40 mm. Hg. Adrenaline 0·02 mg. injected: B.P. rose from 46 to 126 mm. Hg. Right great splanchnic isolated and cut below diaphragm, then stim.: B.P. rose from 48 to 62 mm. Hg. Adrenaline 0·02 mg. injected: B.P. rose from 45 to 146 mm. Hg. Right great splanchnic stim. as before but for 90 sec.: B.P. rose from 52 to 68 mm. Hg and had fallen to 56 mm. by end of 90 sec. stim. Pituitrin 0·2 c.c. (B. and W. infundin) injected: B.P. rose from 48 to 72 mm. Hg; sustained effect. Autopsy revealed no residual cortical tissue. Blood: serum sodium 305 mg./100 c.c.; non-protein nitrogen 165 mg./100 c.c.

Experiment 5. *Cat* 22♂

14. x. 37. Second adrenal removed. Received adrenal cortical extract for 10 days.

24. x. 37. Last injection of adrenal cortical extract. Weight 4·36 kg.

3. ii. 37. Staggers badly when forced to walk. Weight 4·12 kg. Rectal temp. 35·5° C. Ether. Blood drawn from carotid. Urethane 1·3 g. intravenously. B.P. at outset 82 mm. Hg. Right splanchnic nerve[1] stim. continuously for 9 min.: B.P. rose 83 to 120 mm. Hg in

[1] Continuous stimulation of a splanchnic nerve in acutely adrenalectomized or adrenalectomized, extract-treated cats will maintain the B.P. above pre-stimulation level from 35 to 70 min.

90 sec., then declined to 83 mm. Hg by end of 7 min. Right n.m. set up as in Exp. 4, except lever magnification × 15. Right cervical sympathetic nerve stim. continuously: n.m. contracted instantly; maximal recording of lever writing point of 203 mm. attained in a few sec. At about 45 min. and again 65 min. after stim. started, n.m. relaxed to within 60 mm. of starting point. Shifting of electrodes and moistening nerve resulted in immediate return to previous level. 2 hr. 12 min. after stim. started, recorded contraction of n.m. 146 mm. Stim. stopped: n.m. relaxed in 10 min. to 25 mm. above starting point; B.P. then 70 mm. Hg. Blood: haematocrit 46%; potassium 32·6 mg./100 c.c. Autopsy revealed no residual cortical tissue.

In other experiments in which the blood pressure response to splanchnic nerve stimulation was entirely absent it was found that stimulation of the cervical sympathetic produced strong contraction of the n.m. (e.g. Exp. 8, Fig. 5). The actual height of contraction was less than that in other animals at the same stage of adrenal insufficiency as well as in controls. However it should be emphasized that there is so great a variation in the height of contraction obtained in different animals that no direct comparison can be made and, therefore, it is not safe to assert that the response was less than normal though we suspect that may be so in this case.

(c) *Effect of replenishing the depleted blood volume on vascular responses.* There undoubtedly is a marked reduction in blood volume due to plasma water loss in the terminal stage of adrenal insufficiency. (For references, see Swingle, Vars & Parkins [1934].) With this haemoconcentration is associated a marked constriction of peripheral vessels, a condition invariably accompanying the decline of the animal. No doubt these two factors account to some extent for the frequently diminished pressor response to adrenaline which at times may be only about half of that seen in controls acutely adrenalectomized. However, 0·04 mg. adrenaline in these cases (e.g. Exp. 3) will usually elicit a rise of pressure as great as that produced by 0·02 mg. in controls. We have found that, if an animal with adrenal insufficiency is given an intravenous infusion of an amount of saline calculated to restore its blood volume to a normal figure, the effect of 0·02 mg. adrenaline then approximates that elicited by 0·04 mg. prior to the infusion. Restoration of the blood volume, however, does not affect the response to splanchnic nerve stimulation similarly. In three experiments we found no substantial difference before and after the infusion. In one other (Exp. 6) a blood pressure rise of 30 mm. Hg was obtained on stimulation of the right splanchnic following the intravenous injection though none had been obtained on stimulation prior to the administration of the saline. The effect of replacement of the diminished plasma volume with saline on the pressor response to pitressin

or barium chloride is difficult to assess since the second injection of these drugs within a short space of time is vitiated by the effect of the initial injection. These points are demonstrated in the following experiments.

Experiment 6. *Cat* 70 ♂

23. xi. 38. Both adrenals removed. 3·88 kg. Received no adrenal cortical extract.

26. xi. 38. Almost prostrate; weight 3·7 kg. Rectal temp. 34·0° C. Etherized: B.P. 60 mm. Hg, falling gradually. Right splanchnic stim. in abdomen: B.P. rose from 45 to 55 mm. Hg. Adrenaline 0·04 mg. injected: B.P. rose from 45 to 120 mm. Hg. Pitressin 2 units injected: B.P. fell from 52 to 42 mm. Hg, then rose to 60 mm. Hg. Normal saline 40 c.c. injected intravenously over interval of 3 min.: negligible increase in B.P. Right splanchnic stim. in abdomen 9 min. later: B.P. rose from 60 to 74 mm. Hg. Adrenaline 0·04 mg. injected 1½ min. later: B.P. rose from 54 to 194 mm. Hg. Pitressin 2 units injected: no effect. Barium chloride 25 mg. injected: B.P. rose from 25 to 90 mm. Hg. Autopsy revealed no residual cortical tissue. No blood obtained.

Experiment 7. *Cat* 71 ♂

24. xi. 38. Both adrenals removed. Weight 2·34 kg. Received no adrenal cortical extract.

27. xi. 38. Prostrate. Weight 2·20 kg. Rectal temp. 29·0° C. Etherized. B.P. at outset 28 mm. Hg. Right splanchnic stim. in abdomen for 30 sec.: no effect. Adrenaline 0·04 mg. injected intravenously: B.P. rose from 24 to 98 mm. Hg. Pitressin 2 units injected: B.P. rose from 48 to 54 mm. Hg. Normal saline 30 c.c. injected intravenously: B.P. rose from 52 to 88 mm. Hg. Right splanchnic stim. in abdomen 6½ min. later: B.P. rose from 94 to 130 mm. Hg. Adrenaline 0·04 mg. injected intravenously: B.P. rose from 94 to 182 mm. Hg. Pitressin 2 units injected intravenously: B.P. rose from 104 to 112 mm. Hg. Autopsy revealed no residual cortical tissue. No blood obtained.

Unfortunately no haematocrit or other blood analyses were made on these animals. It was felt that the severity of their condition would not safely permit withdrawal of blood. However, the amount of saline given should have restored the blood volume to approximately normal if the haemoconcentration did not exceed the usual degree of change which we have found to be about 15% by haematocrit. Calculation of the blood volume was made on the basis of the figure of 6·2% of body weight obtained by Went & Drinker [1929].

(*d*) *Locus of action of adrenaline.* Since it might be argued that the retention of a pressor effect by adrenaline was due to its effect on the heart alone, we have taken simultaneous records of blood pressure and gut volume during the injection of adrenaline in cats in severe adrenal insufficiency. As shown in Fig. 6, adrenaline caused marked diminution of gut volume due to vasoconstriction which could be seen to be taking place through the glass top of the plethysmograph. We hope to publish more data on this type of study at a later date. However, this result indicates that the dilated splanchnic vessels are still capable of contracting —to adrenaline, at least.

Experiment 8. *Cat* 125♂

17. x. 38. Both adrenals removed. Weight 4·2 kg. Maintained on adrenal cortical extract for 14 days.

1. xi. 38. Adrenal cortical extract discontinued. Weight 4·3 kg.

15. xi. 38. Prostrate. Weight 3·4 kg. Rectal temp. 32° C. Nembutal 30 mg. intravenously. Set up as in Exp. 4, except lever magnification × 15. B.P. at outset 20 mm. Hg. Right splanchnic stim. in abdomen: no effect. Adrenaline 0·04 mg. injected: B.P. rose from 20 to 127 mm. Hg. Right cervical sympathetic stim. 2 min.: height of recorded contraction of n.m. 61 mm. (Fig. 5). Right splanchnic stim.: questionable B.P. effect (Fig. 5). Gut placed in plethysmograph. Adrenaline 0·04 mg. injected: B.P. rose from 24 to 130 mm. Hg; gut volume diminished markedly (Fig. 6). Blood taken from abdominal aorta after death: non-protein nitrogen 118 mg./100 c.c. Autopsy revealed no cortical tissue.

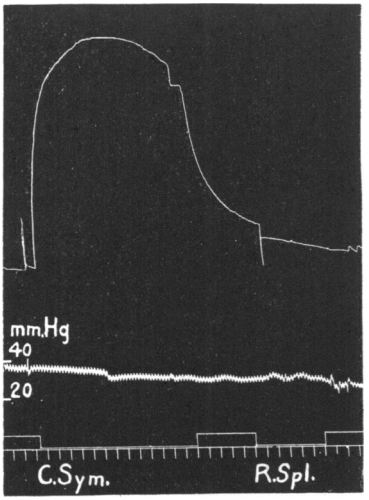

Fig. 5.

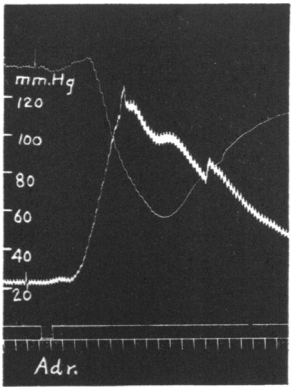

Fig. 6.

Fig. 5. Exp. 8. Nembutal. Terminal stage adrenal insufficiency. Upper tracing of record of contraction of nictitating membrane, lower of B.P. *C. Sym.* right cervical sympathetic chain (cut) stim. peripherally for about 110 sec., not 135 sec. as indicated by signal. *R. Spl.* right splanchnic nerve stim.

Fig. 6. Continuation of tracing in Fig. 5, but instead of n.m. thin line upper tracing now of plethysmograph of gut. *Adr.* 0·04 mg. adrenaline injected. B.P. rose and some 15 sec. later gut volume falls as vessels seen blanching.

(2) *Sympathetic nerve and pressor responses in cortical extract-treated, adrenalectomized cats*

Adrenalectomized cats, maintained in health many weeks by daily injections of cortical extract, show no impairment in the capacity to exhibit a rise in blood pressure in response to splanchnic nerve stimulation or to the injection of pressor drugs. This is demonstrated in Exps. 9 and 10 and in Figs. 7–10.

Experiment 9. *Cat* 25♂

1. xi. 35. Second adrenal removed. Received daily injections of adrenal cortical extract over a period of 90 days, accurate assay of the hormone requirement being made. Had gained 800 g. after 80 days on adrenal cortical extract when a small subcutaneous infection developed at an injection site. A loss of 300 g. occurred in the following 10 days. Though the animal still appeared well, to overcome the infection wholly and render it fit for assay work the administration of an extravagant amount of a.c.e. would have been necessary. Consequently an acute experiment was done on the 90th day.

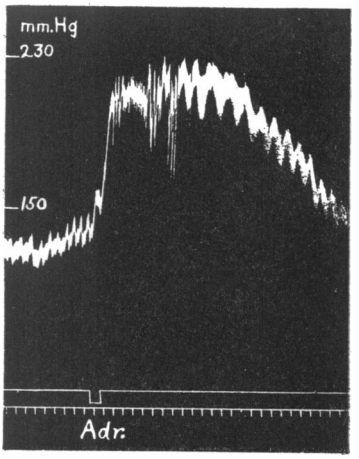

Fig. 7.

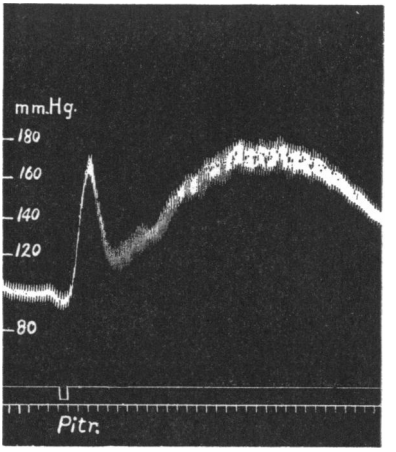

Fig. 8.

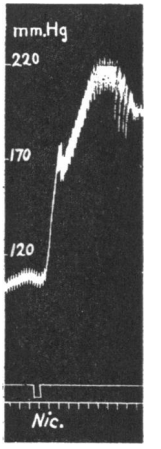

Fig. 9.

Fig. 7. Exp. 9. Nembutal. Extract-treated cat not in adrenal insufficiency. *Adr.* 0·02 adrenaline injected.

Fig. 8. Exp. 9. Continuation of tracing in Fig. 7. *Pitr.* 2 units pitressin injected.

Fig. 9. Exp. 9. Continuation of tracing in Fig. 8. *Nic.* 10 mg. nicotine injected.

30. i. 36. Last adrenal cortical extract injection about 4 hr. previously. Weight 4·43 kg. Nembutal 140 mg. intravenously. Set up for stim. of right cervical sympathetic and recording of right n.m. contraction as for Exp. 4 except for lever which had magnification of ×15. Immediate recorded contraction of n.m. on stim. 80 mm.; after 2 hr. stim. 40 mm.; at end of 3 hr. 30 mm., when stim. terminated. Carotid cannula inserted: B.P. 130 mm. Hg. Adrenaline 0·02 mg. injected: B.P. rose from 144 to 230 mm. Hg, with marked slowing (Fig. 7). Right great splanchnic stim. in abdomen as in Exp. 4—coil distance 9 cm.: B.P. rose from 154 to 190 mm. Hg. Dial 0·5 c.c. intravenously since anaesthesia too light: B.P. fell to 35 mm. Hg; artificial respiration. Adrenaline 0·02 mg. injected: B.P. rose from 28 to 200 mm. Hg; 7 min. later B.P. 102 mm. Hg. Pitressin 0·2 c.c. injected: B.P. rose from 102 to 174 mm. Hg; fell sharply to 124 mm., showing marked slowing of heart; then rose slowly to 184 mm. Hg (Fig. 8). Atropine 5 mg. injected. Nicotine 10 mg. injected 2 min. later: B.P. rose from 110 to 224 mm. Hg (Fig. 9). Autopsy revealed no residual cortical tissue. Blood analysis not done since 8 days previously when values normal.

Experiment 10. *Cat* 38♀

2. xii. 35. Second adrenal removed. History similar to cat in Exp. 9. Had gained 450 g. after 55 days on adrenal cortical extract. Loss of 215 g. in next 6 days associated with two small foci of infection subcutaneously. Acute experiment done on 61st day.

1. ii. 36. Last injection of adrenal cortical extract 18 hr. previously. Weight 3·18 kg. Nembutal 75 mg. intraperitoneally. Set up as in Exp. 9. Immediate recorded contraction of n.m. on stim. 120 mm.; still 78 mm. at end of 3 hr. when stim. terminated. Carotid cannula inserted: B.P. 100 mm. Hg; fell slowly but steadily. Right great splanchnic stim. in abdomen—coil distance 12 cm.: B.P. rose from 70 to 92 mm. Hg; sustained. Coil distance 9 cm.: B.P. rose from 62 to 120 mm. Coil distance 6 cm.: B.P. rose from 54 to 114 mm. Hg.

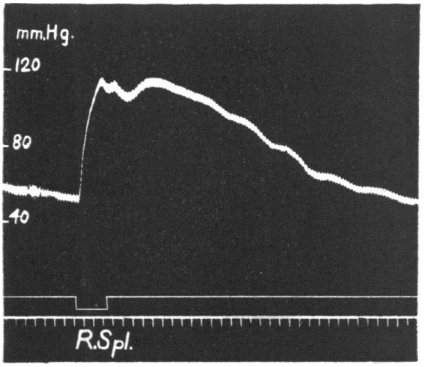

Fig. 10. Exp. 10. Nembutal. Extract-treated cat not in adrenal insufficiency. *R. Spl.* right splanchnic nerve stim.

(Fig. 10). Pituitrin 0·2 c.c. (B. and W. infundin) injected: B.P. rose from 48 to 132 mm. Hg. Atropine 5 mg. injected. Nicotine 10 mg. injected 3½ min. later: B.P. rose from 64 to 144 mm. Hg. Adrenaline 0·02 mg. injected: B.P. rose from 40 to 166 mm. Hg; no slowing. Autopsy revealed no residual cortical tissue. Blood: non-protein nitrogen 46·5 mg./100 c.c.

It appears from these experiments that the absence from the circulation, even for a long time, of adrenaline secreted by the adrenal medulla does not impair the responsiveness of the cardiovascular bed nor the capacity of the n.m. to exhibit sustained contraction on stimulation of its nerve. The amount of adrenaline contained in the extract injected did not exceed 4 μg. daily. Elliott [1914] describes finding good vascular responses nine weeks after adrenalectomy in a cat in which prolongation of life was probably due to the accessory cortical tissue found at autopsy [Elliott's Exp. 1]. This is in agreement with our finding.

Certain qualitative differences in blood pressure response were found in the extract-treated animals compared with acutely adrenalectomized controls. The pressor response to splanchnic nerve stimulation was often found to persist considerably longer after cessation of the stimulus than

in acutely adrenalectomized controls. This persistence of a pressor effect was observed by Hoskins & Wheelon [1914] some hours after adrenalectomy in the dog. Vagal slowing after injection of pressor drugs was more marked at times than in controls acutely adrenalectomized.

Discussion

The results of our study of cats dying of adrenal insufficiency agree, in general, with Elliott's findings. We have not investigated stimulation of the pelvic visceral nerve, however. That lowered body temperature might be the cause of vascular paralysis in adrenal insufficiency was considered by Elliott [1914] and dismissed. Our experiments do not indicate any correlation between the temperature of the animal and the vascular responses. Paralysis may be just as severe when the rectal temperature is 35·2° C. as in Exp. 3, as when it is 31·1° C. as in Exp. 4. Conversely, in animals with temperatures of 33 and 34° C. before the final weakness had set in, we have obtained responses to nerve stimulation and to drugs which, though less than normal, were still good. Finally, the same type of paralysis has been observed in dogs in adrenal insufficiency in which the body temperature was little below normal even though the animal was prostrate [Armstrong, Cleghorn & McVicar, 1937, 1939; Cleghorn, Armstrong & Austen, 1938].

Evidence has been submitted that the failure of splanchnic nerve stimulation and pressor drugs to cause the customary rise in blood pressure in the terminal stage of adrenal insufficiency is dependent, at least only to a slight degree, on the marked reduction in blood volume and attendant increase in viscosity of the blood at this stage. In addition to this we have noted (unpublished observations) that the same degree of haemoconcentration and variation in plasma electrolytes may exist just prior to the onset of the final weakness and fall in blood pressure, and yet good pressor responses be found. This is a strong argument against mechanical or electrolyte changes in blood being of more than minor importance. Further evidence against the electrolyte changes in blood or tissues in adrenal insufficiency being directly responsible for the apparent splanchnic nerve paralysis seems to lie in the persisting effectiveness of cervical sympathetic stimulation in animals showing a marked degree of failure in the vascular response to splanchnic nerve stimulation, e.g. Exps. 4 and 8.

Reduction in the rate of circulation of the blood, which has been shown to exist in adrenal insufficiency by Harrop, Weinstein, Soffer &

Trescher [1933] and others, is possibly an important change contributing to the poor vascular responses. In so far as splanchnic nerve stimulation is concerned, this would probably impede only the rate of change in blood pressure if vasoconstriction occurred to the usual degree.

Some light on splanchnic paralysis may be gained by a consideration of post-mortem findings in adrenal insufficiency; the typical picture is described briefly in Exp. 1. In addition to constriction of peripheral vessels, there is evidence of dilatation of many of the vessels in the splanchnic region. The pancreas is congested very obviously, as also are the mesenteric vessels and mucosa of the stomach, small intestine and, at times, of the large bowel as well. These findings have been described by many workers and frequently observed by us. Obviously the customary tonic vasoconstrictor influence exerted by the splanchnic nerves in this region is in abeyance in the presence of lowered blood volume and pressure. This is contrary to what one would expect. That vasoconstriction is not present in the splanchnic region where the capacity for constriction is normally great means that either a central or a peripheral mechanism is at fault. There is evidence that anoxaemia affects carotid sinus pressor reflexes [Gellhorn & Lambert, 1938] and this may be, in part, the reason for the absence of vasoconstriction in the splanchnic region in adrenal insufficiency. Defective function of carotid sinus reflexes can hardly be more than a minor cause of this and, as already pointed out, the low blood pressure for electrical stimulation of the splanchnic nerve fails to elicit a response comparable to that found in controls. Therefore the dilatation of splanchnic vessels must be due to another physiological fault, or faults. The site of this fault might lie in the sympathetic ganglia which are exposed to the same degree of anoxaemia as the carotid sinus. Possibly the low blood pressure *per se* might also contribute to a ganglionic failure. When the blood pressure is below a level of 30–40 mm. Hg spinal reflexes are unobtainable, a fact we have demonstrated in cats in adrenal insufficiency under urethane anaesthesia. It does not necessarily follow that transmission of impulses across sympathetic ganglia simultaneously become defunct: evidence supplied by Schröder [1907] indicates that sympathetic ganglion cells are more resistant to anaemia than cells of the spinal cord or bulb. Certainly, in adrenal insufficiency the superior cervical ganglion is still capable of transmitting impulses even when the blood pressure is below 40 mm. Hg, e.g. Exp. 8. Therefore, unless one postulates that thoracic and abdominal ganglia suffer a degree of deterioration in function not shown by the superior cervical ganglion, it is not possible to explain the splanchnic

nerve failure on the basis of impaired ganglionic function. This seems to be an unjustifiable assumption.

Exhaustion of the chemical transmitter of adrenergic nerve impulses in the splanchnic region seems to be a more likely explanation for the apparent paralysis of the splanchnic nerves than ganglionic failure. Results with nicotine indicate that cardio-accelerator and adrenergic vasomotor nerves other than the splanchnic are also defective in adrenal insufficiency. The fact that the sympathetic fibres supplying the eye in these animals are not affected, at least to anything like the same degree, seems to be at variance with the results of Secker [1938]. This worker found that the n.m. response to repeated stimulation of the cervical sympathetic fails much more quickly in cats whose adrenals have been removed immediately prior to the period of stimulation than in intact animals.

The reason for the virtual paralysis of vasoconstrictor and cardio-accelerator nerves, which in the cat finally results from adrenal insufficiency, is probably dependent on the sustained activity of these nerves. In all likelihood, the stimulus for this activity lies in a diminution of blood volume due to water loss, which occurs early after removal of the influence of adrenal cortical hormone from the body. This loss of water from the blood is progressive and probably leads to reflex vasoconstriction and increase in heart rate by virtue of carotid sinus and other mechanisms. It would not be surprising, therefore, if those adrenergic nerve endings concerned with the maintenance of blood pressure became exhausted while those to the n.m., not having a vasomotor function, were spared. There might also be a reduction in the rate of production or imperfect metabolism of sympathin in the absence of the cortical hormone, which would accelerate the exhaustion of sympathin.

Direct evidence on the amount of sympathin liberated by adrenergic nerve stimulation in adrenal insufficiency is desirable. This we have endeavoured to obtain by utilizing the denervated n.m. as an indicator of circulating sympathin released on stimulation of a suitable nerve. It is hoped to deal with this aspect of the problem in a subsequent publication.

Pitressin and barium chloride would, under normal circumstances, exhibit a powerful influence on the dilated vessels of the splanchnic region, if not elsewhere. Loss of the local effect of these drugs, rather than general circulatory causes, might be the explanation of the loss of their pressor influence. Observations on the dog seem to support this. In this animal, responsiveness to pitressin frequently is lost early in

adrenal insufficiency, in fact but a few hours after adrenalectomy (unpublished observations). It might be argued that, while these drugs and splanchnic nerve stimulation are ineffective in the animal prostrate from adrenal insufficiency, the reason for the retention of a potent pressor effect by adrenaline lies in the cardiac action alone. The demonstration that splanchnic vessels do constrict to adrenaline is conclusive evidence that vasoconstriction contributes to the pressor effect obtained with this drug.

Summary

1. Some effects of stimulation of sympathetic nerves and of the injection of pressor drugs have been investigated in adrenalectomized cats in the terminal stage of insufficiency and in other adrenalectomized cats maintained in health for many weeks by adrenal cortical extract.

2. In the terminal stage of adrenal insufficiency it was found, in confirmation of Elliott, that splanchnic nerve stimulation or the intravenous injection of nicotine had but a negligible pressor effect. The rise in blood pressure following the injection of either pitressin or barium chloride also was much less than in controls and, in some instances, insignificant. Adrenaline, on the contrary, elicited a very marked rise in blood pressure and stimulation of the cervical sympathetic still caused dilatation of the pupil and strong and sustained contraction of the nictitating membrane.

3. The intravenous injection of an amount of saline calculated to restore to normal the blood volume of a cat in adrenal insufficiency may be followed by a greater rise in blood pressure in response to a standard dose of adrenaline than before the infusion, but in three of four experiments the pressor response to splanchnic nerve stimulation was not improved, or only to a negligible extent, by this procedure.

4. The injection of adrenaline in cats in adrenal insufficiency causes visible constriction of splanchnic vessels and an immediate decrease in the volume of the intestine as determined by the plethysmograph.

5. The loss of the adrenal medulla cannot be held responsible for the impaired vascular response in adrenal insufficiency, for animals kept in good condition by cortical extract treatment after adrenalectomy show responses to brief stimuli and to drugs which, though exhibiting certain qualitative differences, compare favourably with results obtained on acutely adrenalectomized controls.

6. It is suggested that an important factor contributing to the terminal low blood pressure and the failure of splanchnic nerve stimula-

tion and the injection of nicotine to cause much rise in blood pressure in the terminal stage of adrenal insufficiency may be exhaustion of the chemical mediator of impulses of cardio-accelerator and vasoconstrictor nerves.

We wish to express our gratitude to Prof. Duncan Graham for his helpful interest in the carrying out of this work. Our thanks are due to Sir Frederick Banting and to Prof. Andrew Hunter for many courtesies. To Prof. C. H. Best we are indebted for a liberal supply of adrenal cortical extract. Acknowledgement is also due to Messrs W. Cowan and R. Wilson for expert technical assistance.

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